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Purification and Characterization of an N^{α}-Acetyltransferase from *Saccharomyces cerevisiae*[*]

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N^{α}-Acetyltransferase, which catalyzes the transfer of an acetyl group from acetyl coenzyme A to the α-NH_2 group of proteins and peptides, was isolated from *Saccharomyces cerevisiae* and demonstrated by protein sequence analysis to be NH_2-terminally blocked. The enzyme was purified 4,600-fold to apparent homogeneity by successive purification steps using DEAE-Sepharose, hydroxylapatite, DE52 cellulose, and Affi-Gel blue. The M_r of the native enzyme was estimated to be 180,000 ± 10,000 by gel filtration chromatography, and the M_r of each subunit was estimated to be 95,000 ± 2,000 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The enzyme has a pH optimum near 9.0, and its pI is 4.3 as determined by chromatofocusing on Mono-P. The enzyme catalyzed the transfer of an acetyl group to various synthetic peptides, including human adrenocorticotropic hormone (ACTH) (1–24) and its [Phe^2] analogue, yeast alcohol dehydrogenase I (1–24), yeast alcohol dehydrogenase II (1–24), and human superoxide dismutase (1–24). These peptides contain either Ser or Ala as NH_2-terminal residues which together with Met are the most commonly acetylated NH_2-terminal residues (Persson, B., Flinta, C., von Heijne, G., and Jornvall, H. (1985) *Eur. J. Biochem.* 152, 523–527). Yeast enolase, containing a free NH_2-terminal Ala residue, is known not to be N^{α}-acetylated *in vivo* (Chin, C. C. Q., Brewer, J. M., and Wold, F. (1981) *J. Biol. Chem.* 256, 1377–1384), and enolase (1–24), a synthetic peptide mimicking the protein's NH_2 terminus, was not acetylated *in vitro* by yeast acetyltransferase. The enzyme did not catalyze the N^{α}-acetylation of other synthetic peptides including ACTH(11–24), ACTH(7–38), ACTH(18–39), human β-endorphin, yeast superoxide dismutase (1–24). Each of these peptides has an NH_2-terminal residue which is rarely acetylated in proteins (Lys, Phe, Arg, Tyr, Val, respectively). Among a series of divalent cations, Cu^{2+} and Zn^{2+} were demonstrated to be the most potent inhibitors. The enzyme was inactivated by chemical modification with diethyl pyrocarbonate and *N*-bromosuccinimide.

TABLE 1

Purification of N^{α}-acetyltransferase from S. cerevisiae

Step	Activity	Protein	Specific activity	Purification	Yield
	units	*mg*	*units/mg*	*-fold*	%
1. Crude extract	30,200	17,700	1.7	1.0	100
2. DEAE-Sepharose (0.2 M KCl)	32,200	3,710	8.7	5.1	107[a]
3. DEAE-Sepharose (0.05–0.5 M KCl)	61,500	1,470	41.8	24.5	204[a]
4. Hydroxylapatite	19,300	53.6	360	210	64
5. DE52-cellulose	12,700	8.58	1,500	870	42
6. Affi-Gel blue	8,160	1.05	7,800	4,600	27

[a] An apparent inhibitor was removed during these chromatographic steps.

Experimental Procedures

Materials

N-Ethylmaleimide (NEM), iodoacetic acid (IAA), iodoacetamide (IAM), dimethyl-(2-hydroxy-5-nitrobenzyl)sulfonium bromide (HNBS$(CH_3)_2$-Br), 2,4,6-trinitrobenzenesulfonic acid (TNBS), N-acetylimidazole, p-chloromercuribenzoate (pCMB), N-bromosuccinimide (NBS), diethyl pyrocarbonate (DEPC), HEPES, CAPS, CHES, DTT, hydroxylamine, bovine serum albumin, protein standards for M_r determinations, 2-mercaptoethanol, human ACTH(11-24), human ACTH(7-38), human ACTH(18-39), Lys-rich and Arg-rich histone (calf thymus), β-endorphin, glucose, sorbitol, lyticase, Bis-Tris, Tris and glycerol (enzyme grade) were from Sigma. DEAE-Sepharose CL-6B, FPLC column (Mono P HR5/5), Polybuffer 74, Sepharose CL-4B were from Pharmacia. DE-52 cellulose was from Whatman. Protein assay reagent (Bradford method), hydroxylapatite (Biogel HT), Affi-Gel Blue gel and SDS-PAGE electrophoresis reagents were from BioRad. [^{3}H] Acetyl coenzyme A was from Amersham, and unlabeled acetyl coenzyme A was from P-L Biochemicals. Reagents and solvents for amino acid analysis and Ready-Solv EP scintillation cocktails were obtained from Beckman. SP membrane was from Cuno Inc. PM-30 membrane was from Amicon. Yeast extract and Bacto-peptone were from Difco. Constant boiling (6 N) HCl and Polybrene were from Pierce. Phenol was from BRL. Microdialyzer was from Health Products. Reagents and solvents for protein sequence analysis were from Applied Biosystems. Reagents for peptide synthesis were obtained from Applied Biosystems, and solvents for peptide synthesis were from Anachem. Boc-amino acids were from Peninsula. All other chemicals were reagent grade or better.

Methods

General

UV measurements were obtained using a Hewlett-Packard 8450A UV spectrophotometer. Protein assays were performed by the method of Bradford (34) using bovine serum albumin as the standard. Radioactive samples were counted on a Beckman LS 3801 scintillation counter.

Enzyme Assay

Enzyme samples of 1-10 µl were added to 1.5 ml Eppendorf tubes containing a reaction mixture of 50 mM HEPES, pH 7.4, 150 mM KCl, 1mM DTT, 25 µM [^{3}H] acetyl coenzyme A (0.5 µCi) and 50 µM ACTH (1-24) with an adjusted final volume of 100 µl. The assay mixture was incubated at 30 °C for 30 min. The reaction was stopped by adding 17 µl of 0.5 M acetic acid and chilled in an ice bath. The reaction samples were filtered through 2.5 cm diameter SP membrane discs, which had been pre-swollen in and then washed with 0.5 M acetic acid on a Millipore 1225 sampling manifold. The membranes were then washed three times in 1 ml of 0.5 M acetic acid to remove the free [^{3}H] acetyl coenzyme A. The partially dried membranes were placed in 10 ml of scintillation cocktail and counted for 1 min. The radioactivity in the control without ACTH added, representing acetylation of endogenous compounds, was subtracted from each sample determination. One unit of activity was defined as the amount of enzyme able to transfer 1 pmol of [^{3}H] acetyl group from [^{3}H] acetyl coenzyme A to ACTH(1-24) under standard enzyme assay conditions defined above. Other synthetic peptides, including human [Phe^2] ACTH (1-24), human ACTH(11-24), human ACTH(7-38), human ACTH(18-39), β-endorphin, ADH I (1-24), ADH II (1-24), human SOD (1-24), yeast SOD (1-24), yeast enolase (1-24) and Lys- and Arg-rich histones (calf thymus),were substituted for human ACTH (1-24) in the assay.

Cell Growth and Storage

1000 liters of yeast culture (TD 71.8) was grown aerobically at 30 °C in YPD medium (1% yeast extract, 2% Bacto-peptone, 2% glucose) in a Chemap AG fermentor (Chemap AG, Volketswil, Switzerland). Cells were harvested when the culture reached an OD_{660nm} of 14, concentrated to 38 liters by Alfa-Laval separation system (Alfa-Laval Separation AB, Tumba, Sweden), and stored at -20 °C with 10% (v/v) glycerol for up to 4 months without loss of activity.

Cell Extraction

Concentrated yeast culture (6 liters) was thawed, and yeast cells were collected by centrifugation at 4000 rpm for 10 min (JS-4.0 rotor, Beckman). The cells (800 g, wet weight) were resuspended in 1 liter of buffer A (50 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, 3 mM DTT, 1 M sorbitol) containing 80 mg of lyticase, and the cell suspension was shaken gently at 30 °C for 45 min. All subsequent steps were carried out at 4 °C. The spheroplasts were collected by centrifugation at 4000 rpm for 10 min (JS-4.0 rotor, Beckman), washed by gentle resuspension in 500 ml of buffer A, collected by centrifugation and resuspended gently in 400 ml of buffer B (10 mM HEPES, pH 7.4, 1.5 mM $MgCl_2$, 10 mM KCl, and 0.5 mM DTT). The spheroplasts were lysed in this hypotonic buffer by fifteen strokes with a tight-fitting pestle and fifteen strokes with a loose-fitting pestle in a Dounce homogenizer, and then cold KCl (2.0 M) was added to give a final KCl concentration of 0.2 M. The homogenate was gently shaken for 30 min, and debris was removed by centrifugation at 14,000 rpm for 45 min (JA 14 rotor, Beckman). The supernatant solution was concentrated to a volume of 60 ml, using a PM-30 ultrafiltration membrane and dialyzed overnight against 8 liters of HDG (20 mM HEPES, pH 7.4, 0.5 mM DTT, 10% (v/v) glycerol and 0.02% NaN_3) buffer containing 0.2 M KCl.

DEAE-Sepharose CL-6B Chromatography

DEAE Sepharose CL-6B was prepared, degassed, and packed into a column (2.5 x 55 cm) following the manufacturer's recommendations. The column was washed with 4 column volumes of HDG buffer containing 0.2 M KCl (for 0.2 M KCl chromatography) or 0.05M KCl (for linear KCl gradient chromatography). The dialyzed supernatant fluid was applied to DEAE Sepharose CL-6B equilibrated with HDG buffer containing 0.2 M KCl. Acetyltransferase activity was eluted with same buffer at 24 ml/h. Fractions (4 ml) were collected, and the fractions containing acetyltransferase activity were pooled (Figure 1) and concentrated to a volume of 50 ml, using a PM-30 ultrafiltration membrane. This concentrated eluate was dialyzed overnight against 2 x 4 liters of HDG buffer containing 0.05M KCl and then applied to another DEAE Sepharose CL-6B column (2.5 x 55 cm) equilibrated in HDG buffer containing 0.05 M KCl. This column was eluted with a linear gradient of 0.05 M (250 ml) to 0.5 M (250 ml) KCl in HDG buffer at 24 ml/h. Fractions (3.65 ml) were collected, and the fractions containing acetyltransferase activity were pooled (Figure 2) and concentrated to a volume of 5 ml, using a PM-30 ultrafiltration membrane.

Hydroxylapatite Chromatography

The concentrated eluate from the second DEAE-Sepharose chromatography was dialyzed overnight against 2 x 4 liters of 0.05 M potassium phosphate buffer, pH 7.4, 0.5 mM DTT, 10% (v/v) glycerol, 0.02% NaN_3 and applied to a hydroxylapatite column (2.5 x 40 cm) equilibrated with the same buffer used for dialysis. The column was eluted with a linear gradient of 0.05 M (200 ml) to 0.5 M (200 ml) potassium phosphate buffer, pH 7.4, containing 0.5 mM DTT, 10% (v/v) glycerol, 0.02% NaN_3 at 24ml/h. Fractions (4.2 ml) were collected, and the fractions containing acetyltransferase activity were pooled (Figure 3) and concentrated to a volume of 2.5 ml, using a PM-30 ultrafiltration membrane.

DE-52 Cellulose Chromatography

The concentrated eluate from the hydroxylapatite chromatography was dialyzed overnight against 2 x 4 liters of HDG buffer containing 0.05 M KCl and then applied to a DE-52 cellulose column (2.5 x 55 cm) equilibrated in HDG buffer containing 0.05 M KCl. The column was eluted with a linear gradient of 0.05 M (250 ml) to 0.5 M (250 ml) KCl in HDG buffer at 24 ml/h. Fractions (3.4 ml) were collected, and the fractions containing acetyltransferase activity were pooled (Figure 4) and concentrated to a volume of 1 ml, using a PM-30 ultrafiltration membrane.

Affi-Gel Blue Gel Chromatography

The concentrated eluate from the DE-52 cellulose chromatography was dialyzed overnight against 4 liters of HDG buffer containing 0.05 M KCl and applied to an Affi-Gel Blue gel column (1.5 x 25 cm) equilibrated in HDG buffer containing 0.05 M KCl. The column was developed with a linear gradient of 0.05 M (150 ml) to 1 M (150 ml) KCl in HDG buffer at 12 ml/h. Fractions (2.1 ml) were collected, and the fractions containing acetyltransferase activity were pooled (Figure 5) and concentrated to a volume of 1 ml, using a PM-30 ultrafiltration membrane.

Sodium Dodecyl Sulfate-Polyacryamide Gel Electrophoresis

A sample of each pool containing acetyltransferase isolated from each purification step was loaded on a SDS-PAGE gel (8%) and electrophoresed, under reducing conditions, as described by Laemmli (35) (Figure 6). For determination of M_r of purified enzyme subunits, myosin (205,000), *E. coli* β-galactosidase (116,000), rabbit muscle phosphorylase (97,000), bovine serum albumin (66,000), egg albumin (45,000) and carbonic anhydrase (29,000) were used as molecular weight standards. Protein bands were stained with Coomassie Brilliant Blue R. The absence of higher M_r bands in lanes 1-3 resulted from protease degradation which occurred during prolonged storage.

Molecular Size Determination

The M_r of the native protein was estimated by comparison to molecular weight standards by gel filtration on Sepharose CL-4B column (2.5 x 96 cm). Partially purified enzyme, purified through the DE52 cellulose chromatography, was applied to the column. The column was eluted with HDG buffer containing 0.2 M KCl at 20 ml/h. The elution volume of the enzyme was determined by A_{280} nm and enzyme activity, and the apparent molecular weight of yeast acetyltransferase was calculated by comparison with the relative elution volumes of protein standards including thyroglobulin (669,000), apoferritin (443,000), β-amylase (200,000), alcohol dehydrogenase (150,000), bovine serum albumin (66,000) and carbonic anhydrase (29,000).

Chromatofocusing on Mono P

Partially purified enzyme, purified through the DE52 cellulose chromatography, was applied to a Mono P (HR 5/5) column equilibrated with 25 mM Tris-Bis buffer (pH 6) and eluted with Polybuffer 74 (pH 3.6) at the flow rate of 1 ml/min at 4 °C. Elution was monitored by A_{280} nm, and 0.5 ml fractions were collected for measurement of pH and enzyme activity.

Amino Acid Analysis

The concentrated eluate from the Affi-Gel Blue gel chromatography was applied to a 7% SDS-PAGE gel of 1.5 mm thickness in a 12 cm well, electrophoresed, and electroeluted as previously described (36). The amino acid composition was determined using a Beckman 6300 Amino Acid Analyzer after 24 hr hydrolysis at 110 °C in 6 N HCl containing 0.1% phenol (37).

Protein Sequence Analysis

Protein sequence analysis of electroeluted acetyltransferase were carried out twice (each ~300 pmole) by using an Applied Biosystems 470A Protein Sequencer and an Applied Biosystems 120A Pth Analyzer (38).

Chemical Modifications

DEPC (liquid, approximately 6.8 M) was diluted to 1 M with cold absolute ethanol. Further dilutions of DEPC were made with 0.1 M Na-phosphate (pH 6.0) containing 1 mM EDTA and 5% ethanol. NBS and succinic anhydride were dissolved in acetone and diluted with distilled water prior to use. NEM, IAA, IAM, ($HNBS(CH_3)_2$-Br), TNBS, and N-acetylimidazole solution were prepared in distilled water. pCMB was prepared in 10 mM NaOH as a concentrated solution and diluted with distilled water prior to use.

The individual inhibition experiments were done at 30 °C by incubating the enzyme with 50 mM HEPES buffers (pH 7.4) containing the various modification reagents, except for ($HNBS(CH_3)_2$-Br) and DEPC which were used with 50 mM Na-phosphate buffer, pH 6.0. After 15 min, each sample aliquot was dialyzed using microdialyzer against 50 mM HEPES, pH 7.4, 150 mM KCl, 1mM DTT at 4 °C for 3 to 4 hr. The enzyme activity was determined under the standard enzyme assay conditions defined above. As a control, the enzyme was incubated separatedly without added reagents.

Peptide Synthesis

Human ACTH (1-24), human [Phe^2] ACTH (1-24), ADH I (1-24), ADH II (1-24), human SOD (1-24), yeast SOD (1-24), yeast enolase (1-24) were synthesized on an Applied Biosystems Model 430A peptide synthesizer and characterized by methods previously described (39).

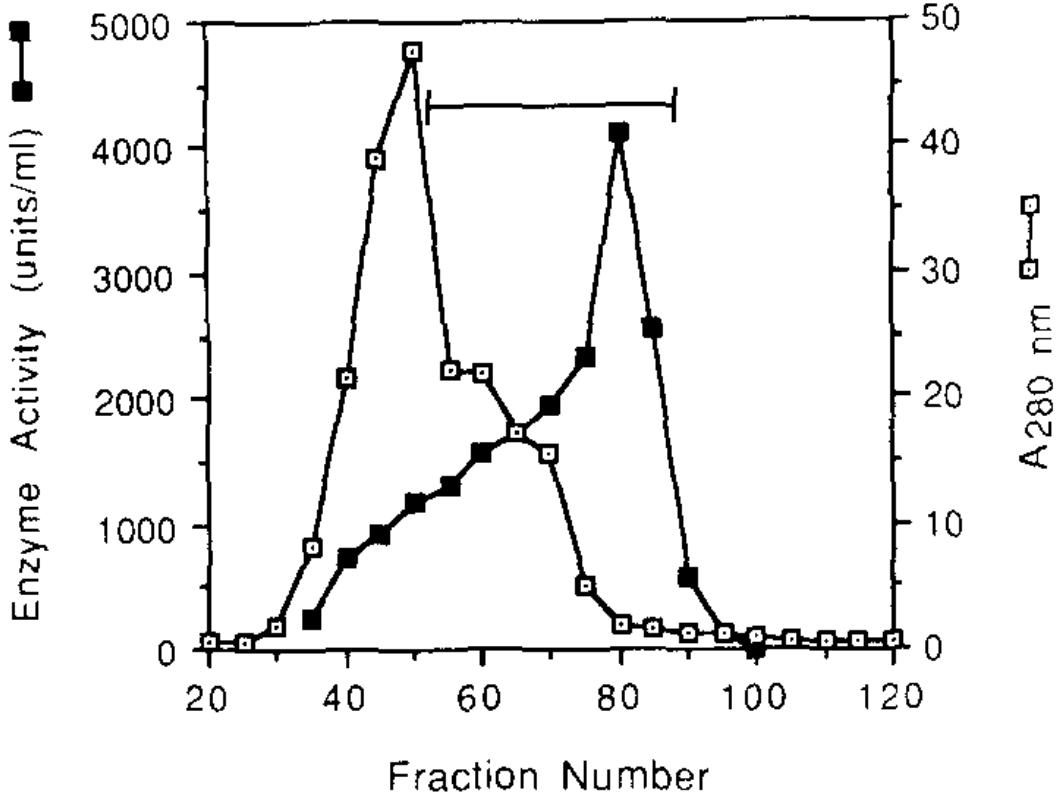

Figure 1. DEAE-Sepharose (0.2 M KCl) chromatography of the yeast N^{α}-acetyltransferase. The supernatant from a yeast extract was applied to a DEAE-Sepharose column and eluted with HDG (20 mM HEPES, pH 7.4, 0.5 mM DTT, 10% (v/v) glycerol and 0.02% NaN_3) buffer containing 0.2 M KCl at 24 ml/h. The A_{280} was measured, and acetyltransferase activity was assayed as described in "Experimental Procedures". Fractions containing acetyltransferase activity were pooled as indicated by horizontal bar.

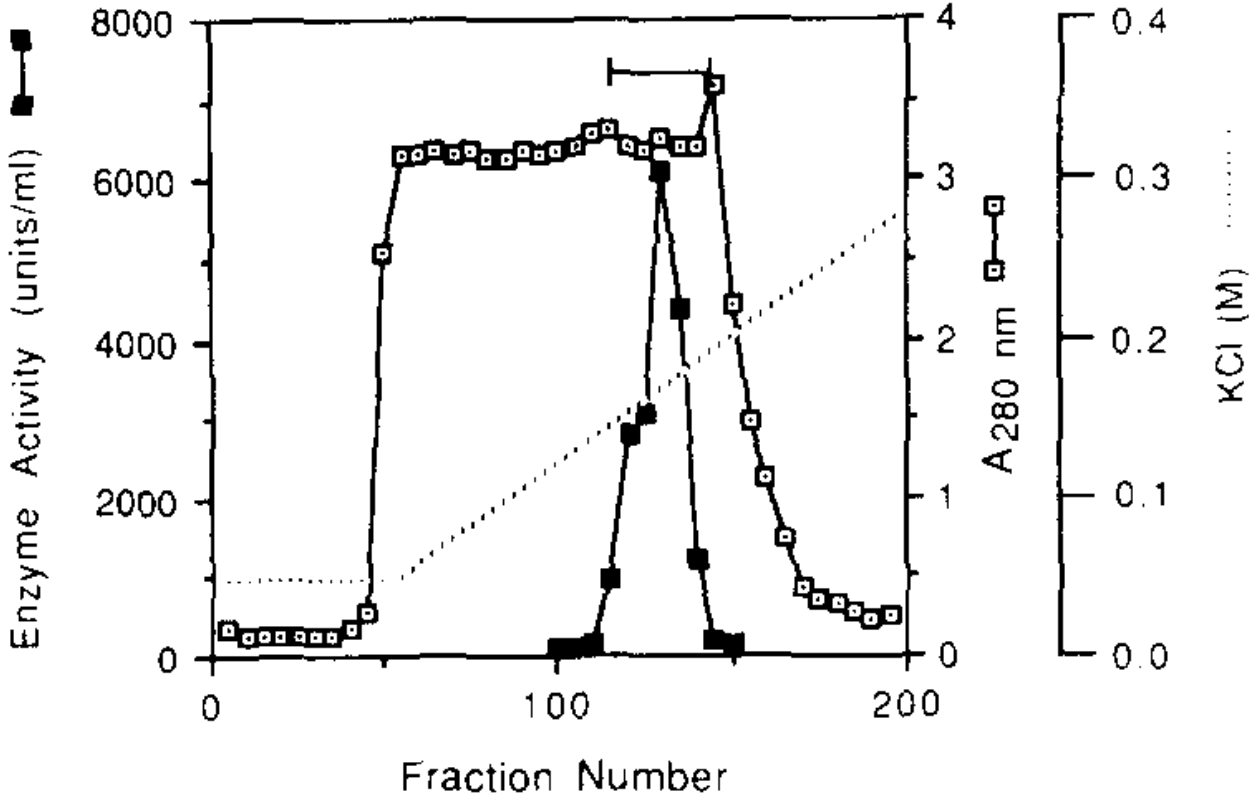

Figure 2. DEAE-Sepharose (0.05 to 0.5 M KCl) chromatography of the yeast N^{α}-acetyltransferase. The acetyltransferase pool from DEAE-Sepharose (0.2 M KCl) was concentrated, dialyzed, and applied to a DEAE-Sepharose column, eluted with a linear gradient of 0.05 M (250 ml) to 0.5 M (250 ml) KCl in HDG buffer at 24 ml/h, and analyzed for A_{280}, conductivity, and acetyltransferase activity, as described in "Experimental Procedures". Fractions containing acetyltransferase activity were pooled as indicated by horizontal bar.

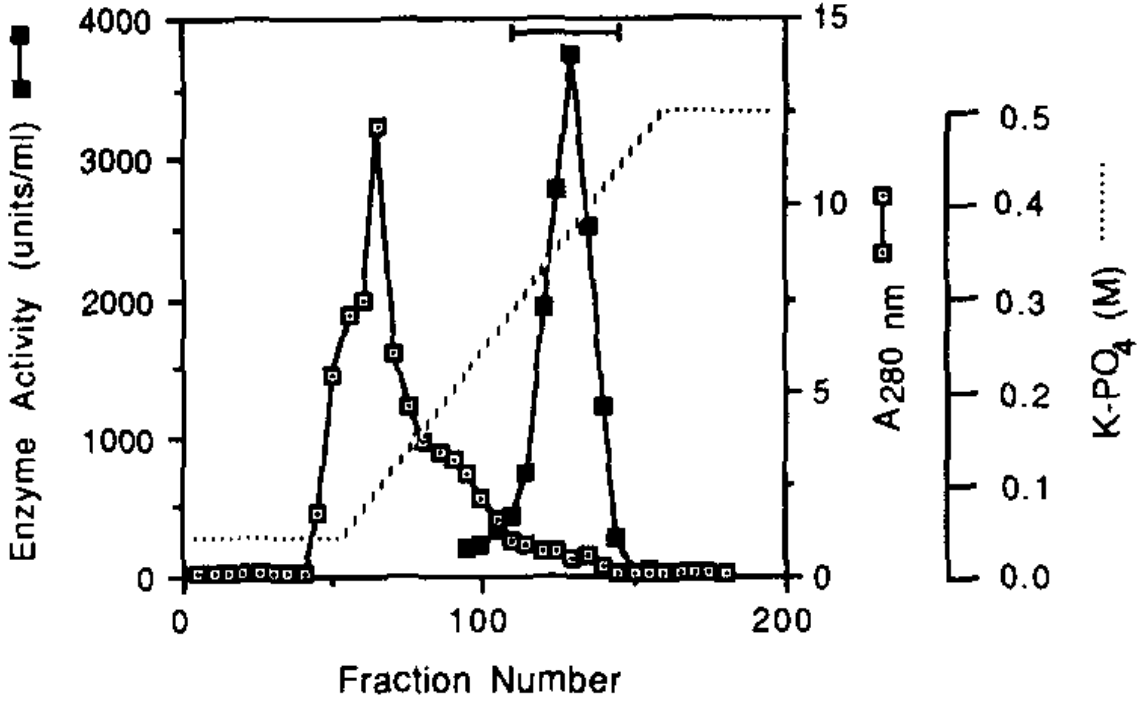

Figure 3. Hydroxylapatite chromatography of the yeast N^{α}-acetyltransferase. The acetyltransferase pool from DEAE-Sepharose (0.05 to 0.5 M KCl) was concentrated, dialyzed, and applied to a hydroxylapatite column, eluted with a linear gradient of 0.05 M (200 ml) to 0.5 M (200 ml) potassium phosphate buffer, pH 7.4, containing 0.5 mM DTT, 10% (v/v) glycerol, 0.02% NaN_3 at 24ml/h, and analyzed for A_{280}, conductivity, and acetyltransferase activity, as described in "Experimental Procedures". Fractions containing acetyltransferase activity were pooled as indicated by horizontal bar.

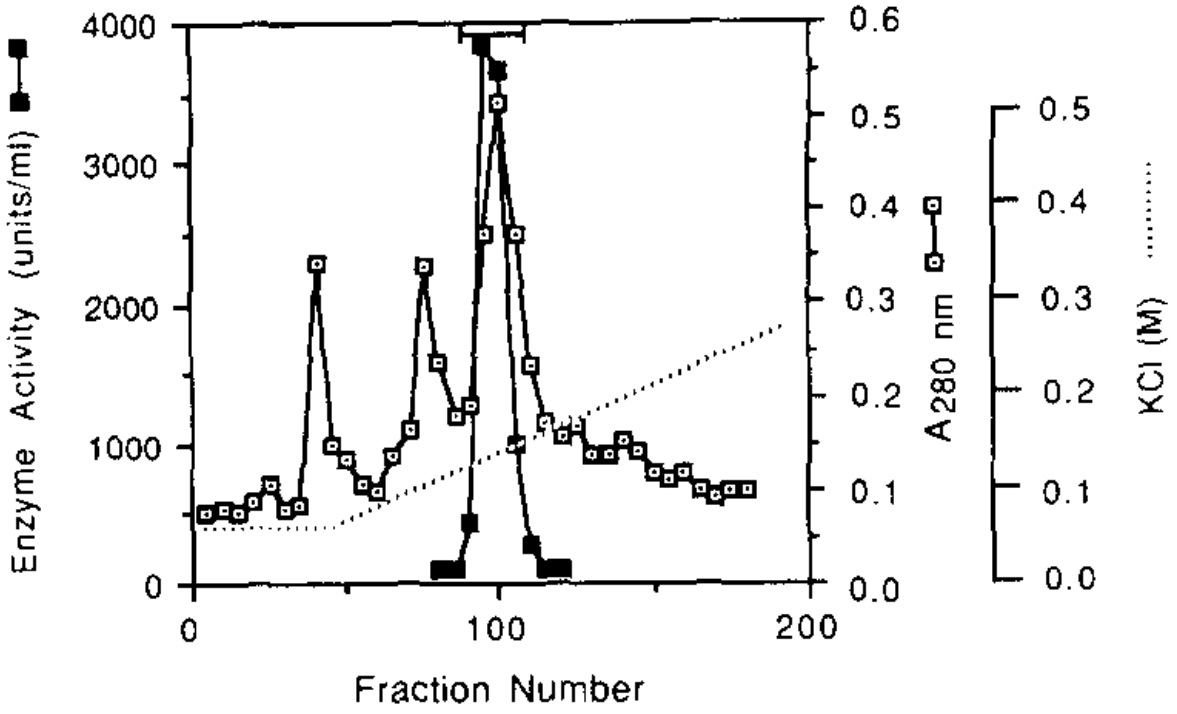

Figure 4. DE52 cellulose chromatography of the yeast N^{α}-acetyltransferase. The acetyltransferase pool from hydroxylapatite was concentrated, dialyzed, and applied to a DE52 cellulose column, eluted with a linear gradient of 0.05 M (250 ml) to 0.5 M (250 ml) KCl in HDG buffer at 24 ml/h. and analyzed for A_{280}, conductivity, and acetyltransferase activity, as described in "Experimental Procedures". Fractions containing acetyltransferase activity were pooled as indicated by horizonatal bar.

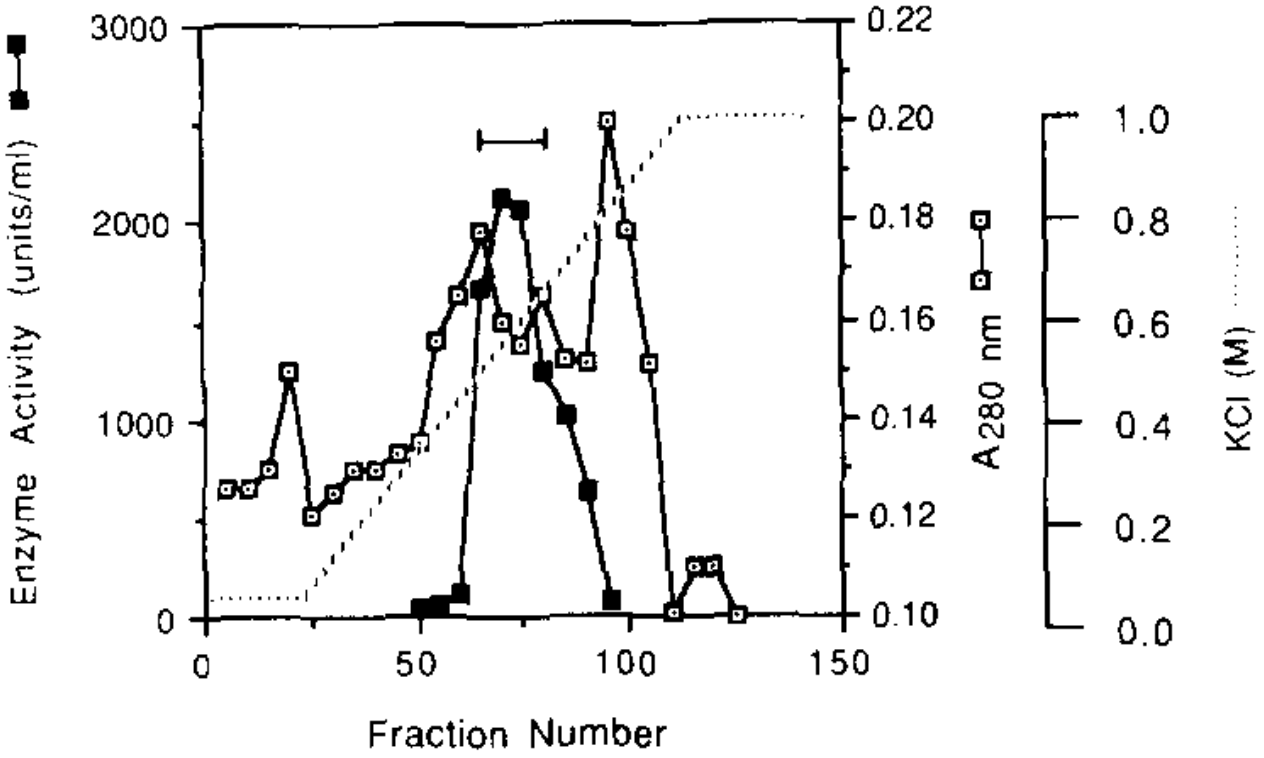

Figure 5. Affi-Gel Blue gel chromatography of the yeast N$^{\alpha}$-acetyltransferase. The acetyltransferase pool from DE52 cellulose was concentrated, dialyzed, and applied to an Affi-Blue gel column, eluted with a linear gradient of 0.05 M (150 ml) to 0.5 M (150 ml) KCl in HDG buffer at 12 ml/h, and analyzed for A_{280}, conductivity, and acetyltransferase activity, as described in "Experimental Procedures". Fractions containing acetyltransferase activity were pooled as indicated by horizontal bar.

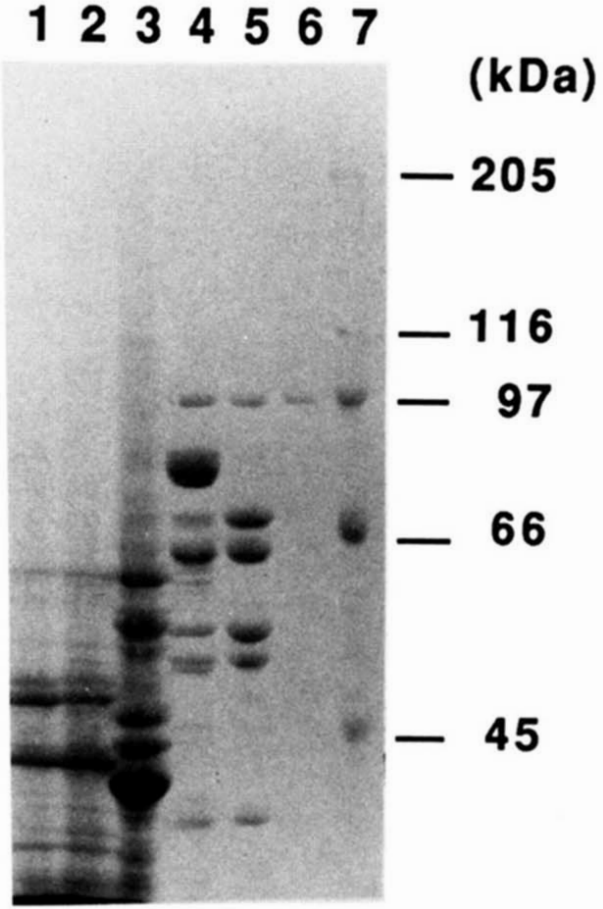

FIG. 6. **Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified yeast acetyltransferase.** The electrophoresis was performed according to the method of Laemmli (30) using an 8% gel. The gel was stained with Coomassie Blue. *Lane 1*, crude extract; *lane 2*, DEAE-Sepharose (0.2 M KCl) pool; *lane 3*, DEAE-Sepharose (0.05 to 0.5 M KCl) pool; *lane 4*, hydroxylapatite pool; *lane 5*, DEAE-cellulose pool; *lane 6*, Affi-Gel blue pool; *lane 7*, molecular weight standards (from the top): myosin (205,000), *E. coli* β-galactosidase (116,000), rabbit muscle phosphorylase (97,000), bovine serum albumin (66,000), and egg albumin (45,000).

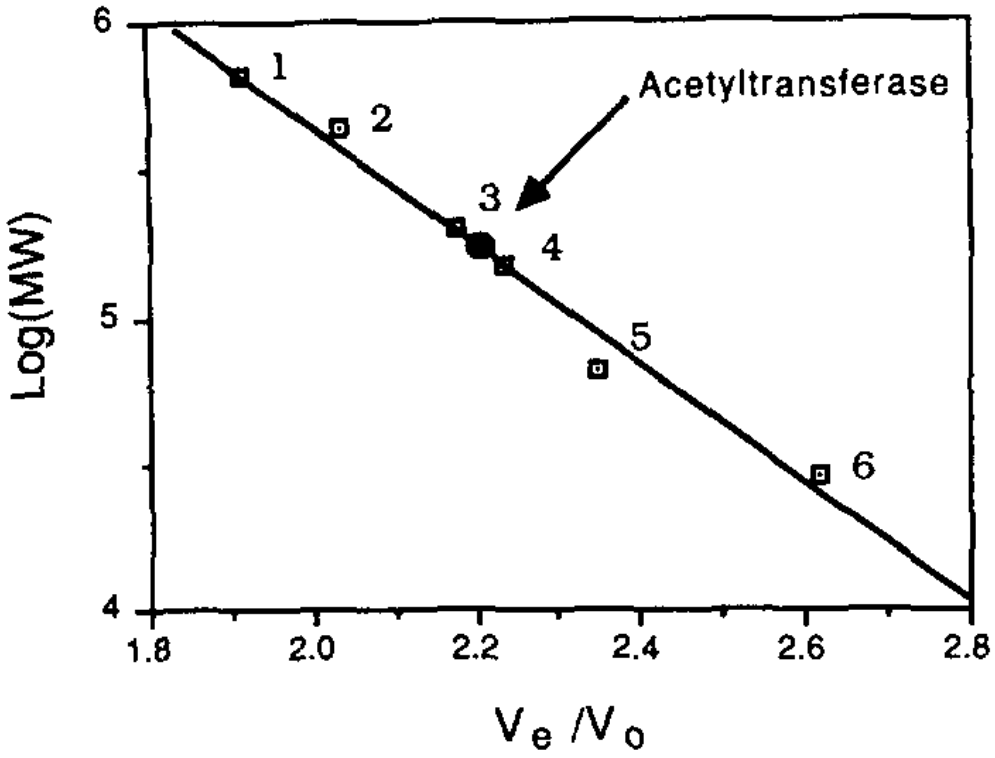

Figure 7. Estimation of the molecular weight of native yeast acetyltransferase by gel filtration. The partially purified enzyme from DE52 cellulose was applied to a Sepharose CL-4B column (2.5 x 96 cm). The elution buffer was HDG buffer containing 0.2 M KCl, and the flow rate was 20 ml/h. The elution volume of acetyltransferase was determined by the standard assay, and the apparent molecular weight was calculated from the relative elution volumes of protein standards including (1) thyroglobulin (669,000), (2) apoferritin (443,000), (3) β-amylase (200,000), (4) alcohol dehydrogenase (150,000), (5) bovine serum albumin (66,000) and (6) carbonic anhydrase (29,000). The elution volume of the enzyme was determined by A_{280} nm and enzyme activity.

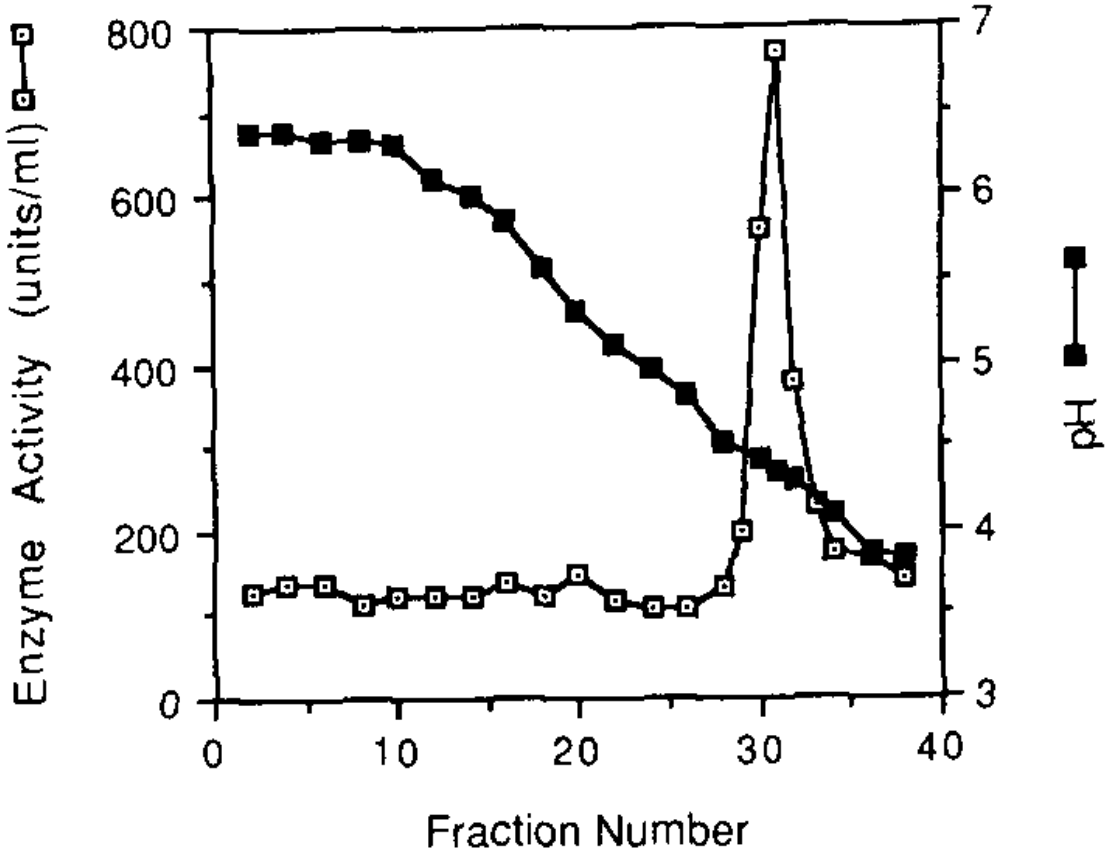

Figure 8. Chromatofocusing of yeast acetyltransferase on a Mono P column. The partial purified enzyme from DE52 cellulose was applied to a Mono P column (HR 5/5) equilibrated with 25 mM Bis-Tris buffer (pH 6) and eluted with Polybuffer 74 (pH 3.6) at the flow rate of 1 ml/min at 4 °C. Elution was monitored by A_{280} nm, and 0.5 ml fractions were collected for measurement of pH and enzyme activity.

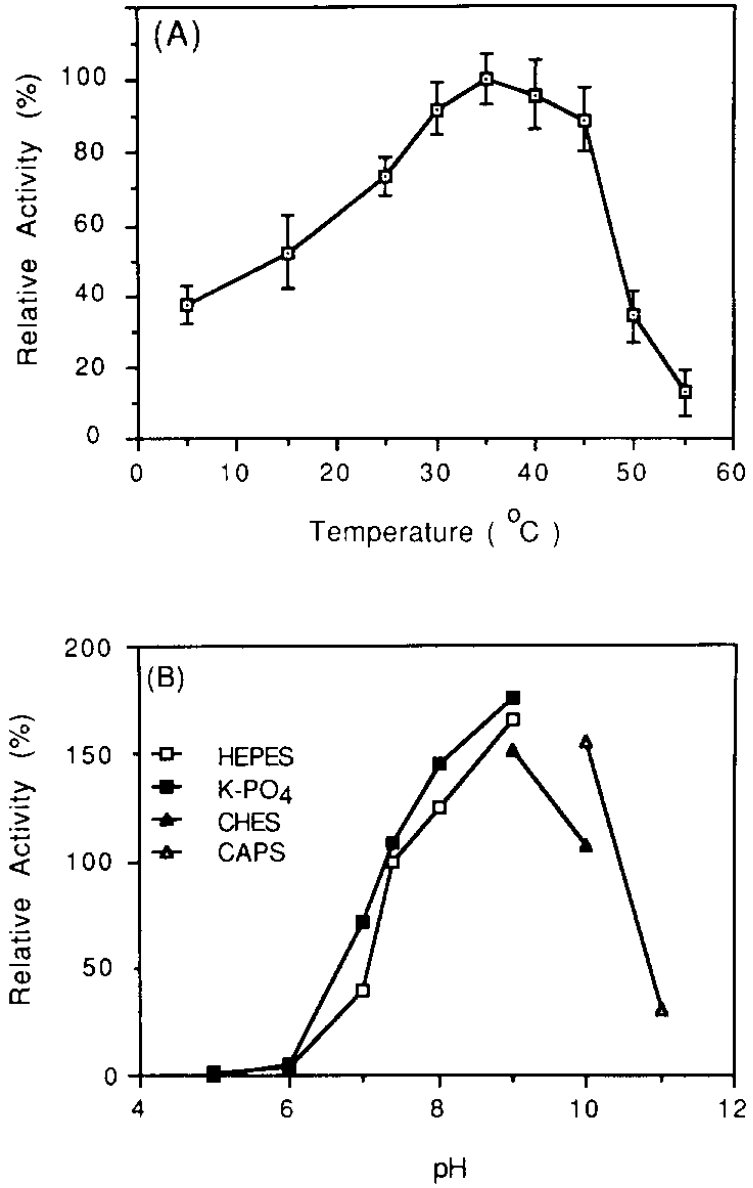

Figure 9. Effect of temperature and pH on yeast acetyltransferase. (A). The specific activity of yeast acetyltransferase for ACTH(1-24) was determined at different temperatures as described in "Experimental Procedures". (B). The specific activity of yeast acetyltransferase for ACTH(1-24) was determined in 50 mM buffers of potassium phosphate, HEPES, CHES, and CAPS buffers of different pH's as described in "Experimental Procedures".

Table 2

Amino Acid Composition of N^{α}-Acetyltransferase from *S. cerevisiae*[a]

Amino Acid	Observed Residues[b]
Asx	103
Thr	24
Ser	43
Glx	96
Pro	32
Gly	39
Ala	61
Val	41
Met	3
Ile	41
Leu	106
Tyr	32
Phe	45
Lys	73
His	12
Arg	37

[a] Purified acetyltransferase was electroeluted from a preparative SDS-PAGE gel. The amino acid composition was determined from six different enzyme preparations using a Beckman 6300 Amino Acid Analyzer after 24 hr hydrolysis at 110 °C in 6 N HCl containing 0.1% phenol (32). Asx = Asp + Asn; Glx = Glu + Gln.

[b] Residue number per subunit of enzyme was calculated on the basis of a M_r = 95,000. No correction was made for the amounts of Ser and Thr destroyed during the 24 hr hydrolysis. Cys and Trp were not determined.

Table 3

Effect of Divalent Cations on Enzyme Activity of N^{α}-Acetyltransferase from *S. cerevisiae*[a]

Salt added	Enzyme Activity (%)		
	Concentration (mM)		
	1	0.1	0.01
None	100	---	---
$CaCl_2$	100	---	---
$MgCl_2$	99	120	---
$MgSO_4$	120	120	---
$MnCl_2$	28	43	84
$CoCl_2$	20	60	86
$CdCl_2$	12	42	90
$FeSO_4$	12	53	78
$CuSO_4$	0	0	58
$ZnSO_4$	0	0	40

[a] Yeast acetyltransferase was incubated in the presence of various divalent cations at a room temperature for 5 min in 50 mM HEPES, pH 7.4 containing 1 mM DTT. The enzyme activity was determined under standard assay conditions using ACTH (1-24) (N = 3), as described in "Experimental Procedures".

Table 4

Effect of Protein Modification Reagents on Enzyme Activity of N^{α}-Acetyltransferase from *S. cerevisiae*[a]

Reagent Added[b]	Concentration (mM)	Enzyme Activity (%)
None		100.0
DEPC	0.5	52
	5.0	1.6
NBS	0.5	30
	5.0	0.5
HNBS$(CH_3)_2$-Br	1.0	82
	10.0	70
2-mercaptoethanol	10.0	110
DTT	10.0	110
NEM	1.0	92
	10.0	13
IAA	1.0	100
	10.0	73
IAM	1.0	98
	10.0	63
pCMB	1.0	100
	10.0	55
TNBS	1.0	94
	10.0	76
Succinic anhydride	1.0	94
	10.0	71
N-acetylimidazole	1.0	100
	10.0	63

[a] Yeast acetyltransferase was incubated with each reagent at 30 °C for 15 min, dialyzed against 50 mM HEPES, pH 7.4, 150 mM, 1 mM DTT at 4 °C for 3 to 4 hr. The enzyme activity was determined under standard assay conditions using ACTH (1-24) (N = 3), assayed as described in "Experimental Procedures".

[b] Abbreviations: DEPC, diethyl pyrocarbonate; NBS, N-bromosuccinimide; HNBS$(CH_3)_2$-Br, dimethyl-(2-hydroxy-5-nitrobenzyl)sulfonium bromide; NEM, N-ethylmaleimide; IAA, iodoacetic acid; IAM, iodoacetamide; pCMB, p-chloromercuribenzoate; TNBS, 2,4,6-trinitrobenzenesulfonic acid.

TABLE 5

Relative activity of yeast acetyltransferase for the N^{α}-acetylation of synthetic peptides and histones

Substrate	Activity[a]
	%
ACTH(1–24) Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro	100 ± 5
[Phe^2] ACTH(1–24) Ser-Phe-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro	90 ± 9
ACTH(11–24) Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro	0
ACTH(7–38) Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu	0
ACTH(18–39) Arg-Pro-Val-Lys-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu	0
β-Endorphin (human) Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ilu-Ilu-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu	2 ± 2
Alcohol dehydrogenase I(1–24) (yeast) Ser-Ile-Pro-Glu-Thr-Gln-Lys-Gly-Val-Ile-Phe-Tyr-Glu-Ser-His-Gly-Lys-Leu-Glu-Tyr-Lys-Asp-Ile-Pro	101 ± 5
Alcohol dehydrogenase II(1–24) (yeast) Ser-Ile-Pro-Glu-Thr-Gln-Lys-Ala-Ile-Ile-Phe-Tyr-Glu-Ser-Asn-Gly-Lys-Leu-Glu-His-Lys-Asp-Ile-Pro	102 ± 4
Superoxide dismutase(1–24) (yeast) Val-Gln-Ala-Val-Ala-Val-Leu-Lys-Gly-Asp-Ala-Gly-Val-Ser-Gly-Val-Val-Lys-Phe-Glu-Gln-Ala-Ser-Glu	0
Superoxide dismutase(1–24) (human) Ala-Thr-Lys-Ala-Val-Cys-Val-Leu-Lys-Gly-Asp-Gly-Pro-Val-Gln-Gly-Ser-Ile-Asn-Phe-Glu-Gln-Lys-Glu	86 ± 6
Enolase(1–24) (yeast) Ala-Val-Ser-Lys-Val-Tyr-Ala-Arg-Ser-Val-Tyr-Asp-Ser-Arg-Gly-Asn-Pro-Thr-Val-Glu-Val-Glu-Leu-Thr	4 ± 2
Histone (lysine-rich) (calf thymus)	0
Histone (arginine-rich) (calf thymus)	0

[a] Data reported as mean activity ± S.D. (n = 3–5).